Insulin Crystals

VI. The Anisotropic Growth of Insulin Crystals

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Rhombohedral pig insulin crystals are formed in a medium containing Indian ink. Growth of the isolated black crystals is continued in a non-coloured medium. It is observed that insulin is not deposited on three of the faces meeting in one of the obtuse vertices, whereas the three opposite faces move outwards with equal rates of growth. The rate is proportional to $(c_s - c)^2$ where c_s is the actual and c the final insulin concentration of the medium. This anisotropy of growth indicates asymmetry of the atomic configuration within the unit-cell.

The purpose of the present investigation is to examine the linear rates of deposition of insulin on the faces during crystallization. Perfect rhombohedral single crystals are obtained when pure pig insulin is crystallized from a citrate buffer and when beef insulin is crystallized from a buffered solution of sodium chloride¹. The crystals are geometrically similar, as the faces of a growing crystal remain equal in size. Hence, the dimensions of a crystal may be defined by one parameter only, *e. g.* by the largest diagonal, l , in the crystal projection on a plane through one of the faces². Previous experiments demonstrate that during crystallization in a 7 % saline

$$\frac{dl}{dt} = \text{const.} (c_s - c)^2 \quad (1)$$

where c_s is the actual and c the final concentration of dissolved insulin³. The linear rate of growth, dl/dt , was found to be independent of the crystal size.

THEORY

The three faces meeting in one of the obtuse vertices are called A, B and C, and the opposite faces A_0 , B_0 and C_0 where A is parallel to A_0 , etc. The growth of a crystal proceeds as insulin is deposited on the faces which thereby move outwards in directions perpendicular to the faces. The rates of movement

caused by deposition are h_a , h_{A_1} , etc. Let the distances between parallel faces be a , b and c , respectively. Then the rates of increase of these distances are

$$\frac{da}{dt} = h_a = h_A + h_{A_1} \quad (2a)$$

$$\frac{db}{dt} = h_b = h_B + h_{B_1} \quad (2b)$$

$$\frac{dc}{dt} = h_c = h_C + h_{C_1} \quad (2c)$$

As during growth

$$a = b = c \quad (3)$$

it follows that

$$h_a = h_b = h_c = h = \text{const.} \quad \frac{dl}{dt} \quad (4)$$

where h symbolizes the common rate of increase of the distances between parallel faces. The trigonal symmetry implicates that the atomic structure of the three faces is identical. Hence, it is assumed that

$$h_A = h_B = h_C = H \quad (5)$$

and

$$h_{A_1} = h_{B_1} = h_{C_1} = H_0 \quad (6)$$

It follows from eqns. (2), (4), (5) and (6) that

$$h = H + H_0. \quad (7)$$

EXPERIMENTAL

It has been observed that when small (5μ) seed crystals are allowed to grow into bigger (30μ) crystals the seed particles remain in one of the obtuse vertices of the growing crystal⁴. If the rate of deposition of insulin is the same on all six faces then the seeds are expected to be enclosed in the center of the crystal. The observation reveals that insulin is not deposited on the three faces meeting in one of the obtuse vertices. This peculiarity of growth has now been substantiated by staining crystals during the first period of their growth. Indian ink is suitable for the purpose as it does not diffuse within the crystal body.

Pork insulin was recrystallized from a medium containing 0.5 % insulin, 0.08 % ZnCl_2 , 0.05 M sodium citrate, 15 % (v/v) acetone and 1 % (v/v) Indian ink (Pelikan No. 541), pH = 6.3. The clear solution was stirred slowly mechanically. During crystal growth particles of Indian ink became enclosed in the crystal bodies and stained them. The crystals were filtered off and one part allowed to continue growing in a medium of the original composition but without Indian ink. The other part was grown in a crystallization medium containing 1.7 % insulin, 0.014 % Zn^{++} (total Zn), 0.1 M sodium acetate, 7 % NaCl, pH = 5.5. In both cases the weight of stained crystals was 10 % of the weight of insulin present.

Finally, the two crystal suspensions were fixed in gelatine in order to prevent sedimentation of all the crystals on their faces during the microscopical inspection. This revealed that the original black part of the crystal is lying in one of the obtuse vertices of the large crystal and not in the center (Fig. 1).

Fig. 1. Stained crystals after growth in saline medium (300 $\times$).

CONCLUSION

These experiments demonstrate that insulin is only deposited on the three faces meeting in one of the obtuse vertices of the crystal. The linear rate of deposition does not vary between these faces. Thus, in eqn. (7) either H or H_0 is zero. It is concluded that the atomic structure of the three moving faces is different to that of the immobile faces. The structural difference also pertains to the unit-cells as they are all identically orientated within the crystal. In other words, when the unit-cell is turned upside down so as to invert the trigonal axis, it is impossible to obtain the original atomic configuration by rotating about this axis.

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